[0041] FIG. **6** is a thermal property curve constructed from data generated from the thermal disassociation of a double stranded oligonucleotide.

[0042] FIG. **7** is a schematic depiction of a microfluidic device in accordance with the invention.

[0043] FIG. **8** shows the variation in time of a measured temperature and of a fluorescent signal.

[0044] FIGS. **9**A-**9**C schematically depicts a portion of a microfluidic system that may be employed in some embodiments of the invention.

[0045] FIG. **10** shows a microfluidic device capable of performing continuous flow PCR followed by melting curve analysis of the amplicons.

[0046] FIGS. **11**A-**11**D show the portion of an integrated system that interfaces with the microfluidic device of FIG. **10**.

[0047] FIGS. **12**A-**12**B show example data from a DNA thermal denaturation (thermal melt) experiment obtained using the microfluidic device shown in FIG. **10**.

DETAILED DESCRIPTION OF THE INVENTION

[0048] Methods and devices in accordance with the invention are capable of rapidly characterizing a variety of biological materials via the generation of molecular melt curves. For example, the molecular melt curve of a double stranded DNA molecule can provide information about the number of base pairs in the molecule, the GC content, and the amount of variation from ideal Watson-Crick base pairing. A molecular melt curve also can be used to indicate the degree of binding between one or more test molecules and a target molecule. "Binding" includes not only, e.g., receptor-ligand interactions, but also, e.g., nucleic acid-nucleic acid hybridization interactions and can include both specific and nonspecific interaction. If the test molecules do bind to the target molecule, then their binding can be quantified by the invention. The methods and devices herein are flexible and can be applied to many different types of compounds and molecules. For example, both the target molecule and the test molecules can be any one or more of, e.g., a protein (whether enzymatic or not), an enzyme, a nucleic acid (e.g., DNA and/or RNA, including, single-stranded, double-stranded, or triple-stranded molecules), a ligand, a peptide nucleic acid, a cofactor, a receptor, a substrate, an antibody, an antigen, a polypeptide, monomeric and multimeric proteins (either homomeric or heteromeric), synthetic oligonucleotides, portions of recombinant DNA molecules or chromosomal DNA, portions or pieces of proteins/peptides/receptors/etc. that are capable or having secondary, tertiary, or quaternary structure, etc. The target molecule also optionally interacts with, e.g., co-enzymes, co-factors, lipids, phosphate groups, oligosaccharides, or prosthetic groups.

[0049] Briefly, the methods and devices of the invention enable the construction of and comparison of molecular melt curves. Molecular melt curves are alternatively described as "thermal melting curves", "thermal melt curves", "thermal property curves", "thermal denaturation curves" or "thermal profile curves." Accordingly, an analysis involving the generation of molecular melt curve can also be described as a molecular melt analysis, a thermal melting analysis, a thermal melt analysis, a thermal property analysis, a thermal denaturation analysis, or a thermal profile analysis. In such an analysis, a sample of a target molecule, or target molecules, to be tested is flowed into one or a number of microchannels in a microfluidic device. Optionally, the target molecule is then contacted with one or more test molecules that are screened for possible binding capability with the target molecule and/or with an indicator such as a fluorescence indicator dye or molecule. Optional embodiments of the present invention allow for multiple configurations of, e.g., heat application, flow speed, reagent composition, binding conditions, and timing of all the multiple variants involved.

[0050] Once the test molecule interacts with the target molecule and/or labeling compound, the present invention sets the reaction conditions, in a controllable manner, to a desired temperature (either continuously over a range of temperatures or non-continuously to discrete temperature points). Selected physical properties of the molecules are measured in the microfluidic device and thermal property curves produced from the measurements. The thermal property curves are based upon, e.g., the temperature induced denaturation or unfolding that occurs when the molecules are subjected to heat. Denaturation can include, e.g., loss of secondary, tertiary, or quaternary structure by means of uncoiling, untwisting, or unfolding, disassociation of nucleic acid strands, etc. When target and test molecules bind to one another, e.g., as with receptor-ligand interactions, the conformation of the target molecule is stabilized and the pattern of the temperature induced denaturation is altered or shifted. Comparison of the thermal property curve derived from heating just the target molecule, with the thermal property curve derived from heating the target molecule and test molecule(s) in combination, allows the determination and quantification of any binding between the target molecule and the test molecule(s). The adaptability of the current invention optionally allows both thermal property curves to be run simultaneously in the microfluidic device, as well as optionally running multiple configurations of the binding assay simultaneously (e.g., with different reaction parameters, such as pH, temperature gradient(s), etc.).

[0051] Numerous types of molecules can be assayed by the methods, devices, and systems of the present invention. For example, protein-protein binding reactions can be examined, including, e.g., receptor-ligand, antibody-antigen, and enzyme-substrate interactions. Additionally, interactions between, e.g., amino acid based molecules and nucleic acid based molecules can be examined. Similarly, artificial molecules such as peptide nucleic acids (PNAs) can be monitored, e.g., in interactions of the PNAs with nucleic acids or other molecules. Also, screening for interactions between hydridization probes and nucleic acids, e.g., comprising single nucleotide polymorphisms (SNPs), can be accomplished through use of the current invention. For examples of types of molecular interactions optionally assayed by the invention, see, e.g., Weber, P. et al., (1994) "Structure-based design of Synthetic Azobenzene Ligands for Streptavidin" *J Am Chem Soc* 16:2717-2724; Brandts, J. et al., (1990) *American Laboratory,* 22:3041+; Gonzalez, M. et al., (1997) "Interaction of Biotin with Streptavidin", *J Biol Chem,* 272(17): 11288-11294; Chavan, A. et al., (1994) "Interaction of nucleotides with acidic fibroblast growth factor (FGF-1) *Biochem,* 33(23):7193-7202; Morton, A. et al., (1995) "Energetic origins of specificity of ligand binding in an